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Distinct physiological and metabolic reprogramming by highbush blueberry (*Vaccinium corymbosum*) cultivars revealed during long-term UV-B radiation

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Despite the Montreal protocol and the eventual recovery of the ozone layer over Antarctica, there are still concerns about increased levels of ultraviolet-B (UV-B) radiation in the Southern Hemisphere. UV-B induces physiological, biochemical and morphological stress responses in plants, which are species-specific and different even for closely related cultivars. In woody plant species, understanding of long-term mechanisms to cope with UV-B-induced stress is limited. Therefore, a greenhouse UV-B daily course simulation was performed for 21 days with two blueberry cultivars (Legacy and Bluegold) under UV-B_{BE} irradiance doses of 0, 0.07 and 0.19 W m^{-2}. Morphological changes, photosynthetic performance, antioxidants, lipid peroxidation and metabolic features were evaluated. We found that both cultivars behaved differently under UV-B exposure, with Legacy being a UV-B-resistant cultivar. Interestingly, Legacy used a combined strategy: initially, in the first week of exposure its photoprotective compounds increased, coping with the intake of UV-B radiation (avoidance strategy), and then, increasing its antioxidant capacity. These strategies proved to be UV-B radiation dose dependent. The avoidance strategy is triggered early under high UV-B radiation in Legacy. Moreover, the rapid metabolic reprogramming capacity of this cultivar, in contrast to Bluegold, seems to be the most relevant contribution to its UV-B stress-coping strategy.

Abbreviations – $^1O_2$, singlet oxygen; ^{3}Chl, triplet state of chlorophyll; AAPH, 2,2′-azobis (2-amidinopropane) dihydrochloride; AUC, area under decay curve; Ax, antheraxanthin; DEPS, de-epoxidation state of the xanthophyll-cycle pigments; ETR, electron transport rate; FL, flavonoid; Fv/Fm, maximum quantum yield; LP, lipid peroxidation; Lt, lutein; MDA, malondialdehyde; NPQ, non-photochemical quenching; Nx, neoxanthin; NPK, Nitrogen, Phosphorus and Potassium; ORAC, oxygen radical antioxidant capacity; PAR, photosynthetically active radiation; Phe, phenolic acid; ϕPSII, effective quantum yield of PSII; RGR, relative growth rate; ROS, radical oxygen species; RSC, ROS scavenging capacity; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; TA, total anthocyanin; TBARS, thiobarbituric acid reacting substances; TC, total carotenoid; TE, Trolox equivalent; TP, total phenol; UAC, UV-absorbing compound; Vx, violaxanthin; Zx, zeaxanthin; βCa, β-carotene.

Introduction

Many regions in the Southern Hemisphere have been severely impacted by the decrease in the ozone layer, which has led to higher doses of UV-B radiation (between 280 and 320 nm) reaching the earth's surface (Mckenzie et al. 2007). The interaction between ozone depletion and climate change can modify expectations regarding the future of UV-B scenarios, which maintain that this issue is a current concern (Ballare et al. 2011). Although UV-B radiation represents only a small fraction of the total solar spectrum, it has disproportionately large photobiological effects on plants because of its high energy, which has been extensively reviewed (Jansen et al. 1998, Kakani et al. 2003, Lidon et al. 2012, Kataria et al. 2014). A common negative effect of UV-B is the enhancement of reactive oxygen species (ROS) production generating oxidative stress, lipid peroxidation of biomembranes and organelles, including chloroplasts (He et al. 1994, Landry et al. 1995, Lidon and Ramalho, 2011). It has been demonstrated in *Arabidopsis* plants that short-term responses to UV-B exposure occur only at the level of primary metabolites (Kusano et al. 2011). This suggests that these responses allow the cell to promote later production of UV-B-absorbing secondary metabolites. In addition to metabolite reprogramming, UV-B irradiation damages the photosynthetic apparatus, negatively affecting its function through direct effects on both photosystem I and II (PSI and PSII) and consequently limiting the carbon assimilation (Pfündel 2003, Lidon and Ramalho 2011). Furthermore, it has been shown that UV-B radiation reduces the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and/or its protein amount in some plant species such as *Oryza sativa* (Takeuchi et al. 2002, Fedina et al. 2010) and *Canavalia ensiformis* (Choi and Roh 2003), affecting the CO_2 assimilation. This radiation also impacts the stability of the chloroplast ultrastructure, affecting the light harvesting apparatus by inducing changes at the level of photosynthetic pigments (especially chlorophylls) and thus reducing photosynthesis (He et al. 1994, Hui et al. 2013, Kataria et al. 2014).

Carotenoids are known to have a role in UV-B photoprotection in *Arabidopsis* plants (Middleton and Teramura 1993). However, some evidence indicates a non-direct assignment of total carotenoids (TC) in UV-B protection as established in *Avena sativa* (Ruhland et al. 2007) and *Fagopyrum esculentum* (Gaberščik et al. 2002). By contrast, xanthophyll cycle pigments are involved in the dissipation of excess energy in plants (Baroli and Niyogi 2000). Under excessive light, the xanthophyll pigment zeaxanthin (Zx) rapidly undergoes de-epoxidation from violaxanthin (Vx), through the intermediate antheraxanthin (Ax). This reaction is reversed under dark conditions (Jahns and Holzwarth 2012). With respect to UV-B radiation, the participation of the xanthophyll cycle pool is still controversial. In this sense, Bolink et al. (2001) reported that while in *Pisum sativum* the levels of these pigments increased after UV-B exposure, in *Phaseolus vulgaris* they remained unchanged. Moreover, in *P. sativum*, it is suggested that violaxanthin de-epoxidase (VDE) is inhibited by UV-B, resulting in enhanced levels of Vx.

Plants have evolved strategies to counteract the negative effects of UV-B radiation (Solovchenko and Merzlyak 2008). One of these is the accumulation of UV-B absorbing compounds [e.g. secondary metabolites such as phenolic acids (Phe), flavonoids (FL), hydroxycinnamic acid, etc.]. These compounds are accumulated mainly in epidermal cells, screening the amounts of UV-B radiation reaching the photosynthetic leaf tissues. They are considered an efficient avoidance UV-B-resistance mechanism, where the UV-B can cause damage (Li et al. 1993, Landry et al. 1995, Mazza et al. 2000, Kolb et al. 2001). Metabolomic studies among species and genotypes subjected to UV-B have revealed different phenolic compositions and de novo synthesis of molecules (Casati et al. 2011, Kusano et al. 2011). In this context, it has been suggested that among flavonoids, the flavonols subclass is the main group induced under UV-B radiation (Pollastri and Tattini 2011, Zoratti et al. 2014). In general, flavonoids have a strong UV-B absorbance capacity and relatively high antioxidant capacity depending on their structure (Rice-Evans et al. 1996). Nevertheless, some authors assign a major role to this group as UV-B absorbing or photoprotective compounds (Li et al. 1993, Bieza and Lois 2001).

Moreover, as a complement to the role of the UV-B absorbing compounds Phe and FL along with other plant secondary metabolites act as antioxidants through the scavenging of ROS (Rice-Evans et al. 1995, Pietta 2000). According to Solovchenko and Merzlyak (2008) this is a tolerance mechanism. The ROS scavenging capacity (RSC) is primarily attributed to the hydroxyl substituents of Phe and FL, conferring antioxidant, chelating and prooxidant activity (Heim et al. 2002). Nonetheless, flavonoids are often found as glycosylated derivatives in plants, which negatively influences their RSC (Pietta 2000). In addition to the non-enzymatic antioxidant strategies detailed above, enzymatic antioxidants such as superoxide dismutase (SOD) is also involved in UV-B protection in plants. According to Hideg et al. (2002), the dominant ROS produced under UV-B stress is the anion superoxide (O_2^-), which is scavenged by SOD. It has been reported that SOD activity varied among plants

species according to the UV-B dose applied (Agrawal et al. 2009).

South-central Chile, an important agricultural region, has increased levels of UV-B radiation because of the decreasing ozone layer (Huovinen et al. 2006). Thus, many crops are cultivated under high UV-B radiation as is the case of highbush blueberry (*Vaccinium corymbosum*). The high antioxidant attributes of its fruits, as well as its high economic returns, have positioned the blueberry as an important crop in this region (Ribera et al. 2010). Our previous studies have shown different capacities of cultivars to respond to the abiotic stresses such as aluminum and manganese toxicities frequently found in south-central Chile (Reyes-Díaz et al. 2009, 2010, Rojas-Lillo et al. 2014). In these studies, we demonstrated that some *V. corymbosum* cultivars showed contrasting behavior under these abiotic constrains, with Legacy being considered a resistant and Bluegold a sensitive cultivar. However, information about metabolic and antioxidant strategies affected by UV-B radiation in *V. corymbosum* cultivars is still limited. Thus, the present work aimed to determine the effect of increasing doses of UV-B radiation in a long-term treatment in *V. corymbosum* cultivars and evaluate the strategy to counteract the negative effects of this stress. Therefore, plant growth, photosynthetic performance, antioxidants, photoprotective compounds accumulation, lipid peroxidation and metabolic responses to UV-B stress of Legacy and Bluegold were analyzed.

Materials and methods

Plant material and experimental conditions

In this work, two highbush blueberry (*V. corymbosum*) cultivars (Legacy and Bluegold) frequently cultivated in south-central Chile were used. The 2-year-old clone plants were provided by Berries San Luis in south-central Chile (38°29′S, 72°23′W), transported to the Universidad de la Frontera (38°44′S, 72°37′W) and acclimated for 3 months under greenhouse conditions. Prior to the UV-B application, plants were conditioned by washing the roots carefully and placing them in 3 l pots containing an Andisol (volcanic ash soil), a soil substrate typical of the region where this species is cultivated. The soil was previously analyzed for its chemical composition (organic matter, pH, available P, S and N, exchangeable Al, Mn, Mg, Ca, K and Na) according to Sadzawka et al. (2004). Based on the chemical analyzes, soil was fertilized according to the agronomic fertilization for blueberry plants. Thus, the NPK (Nitrogen, Phosphorus, Potassium) ratio of 2:1:1 (3:1.5:1.5 g m^{-2}; Hancock and Hanson, 1986) was applied by fertigation for1 month of pre-UV-B treatments.

Greenhouse irradiation conditions

Three irradiation conditions were used in this study: (1) photosynthetically active radiation (PAR) irradiation without UV-B exposure (−UV-B), (2) PAR plus UV-B, simulating a typical winter daily course of UV-B radiation, which corresponds to low UV-B radiation and (3) PAR plus UV-B simulating a summer daily course of radiation which corresponds to a 30% increase in UV-B intensity over the typical irradiance of UV-B in south-central Chile (De los Rios et al., 2007, De los Rios and Acevedo, 2010). The UV-B radiation was applied via UV-B Q-lamps (Q-Panel 313; Cleveland, Ohio) enclosed in a cellulose diacetate (0.08 mm) filter to remove UV-C radiation from the lamps (Middleton and Teramura 1993). Under this condition, the transmitted photon flux energy was 57% of the total UV-B and only 5.7% UV-A radiation. Thus, it was possible to ensure that the UV radiation applied in this study was mainly biologically effective UV-B. The Q-lamps were suspended 20 cm above the top of plant. The UV-B daily course was carried out automatically by setting individual timers (mod. Temps 24H, Halux®, Santiago, Chile) on each Q-lamp to obtain 2.5 and 5.2 W m^{-2} UV-B irradiance (63.0 and 128.7 kJ m^{-2} day^{-1} UV-B daily dose, respectively). The biologically effective UV-B ($UV\text{-}B_{BE}$ irradiance) was 0.07 and 0.19 W m^{-2} for low and high UV-B treatments, respectively (Fig. S1, Supporting Information). The UV-B radiation range was 280 to 320 nm and measured with a spectroradiometer (Li-COR® 1800, Lincoln, NE). Daily PAR radiation was followed with Li-COR Li-189, with sensor Quantum N° Q32496 (Lincoln, NE). Measurements were taken during the day above the plants tops. The daily PAR was on average 400 μmol photons m^{-2} s^{-1} day^{-1}. All the experiments were performed simultaneously in greenhouse and PAR conditions were the same for all the UV-B levels simulated.

During the assay, the plants were maintained at 24°C (±2°C), relative humidity of 75% (±5%), a photoperiod of 16 h light and 8 h dark. Irrigation was performed every day to maintain water field capacity. The plants remained under different UV-B doses for 7, 14 and 21 days. To minimize any positional effect, the placement of pots with plants was changed randomly every day.

A completely randomized experimental $2 \times 3 \times 3$ design (two blueberry cultivars, three UV-B treatments and three time periods) was used with three replicates per treatment. The experimental units consisted of one plant per pot, giving a total of 54 pots for the assay. Measurements were taken at the beginning of the light period on each harvesting day. Samples were collected from fully expanded leaves from the first upper third top

of the plant, immediately frozen in liquid nitrogen and stored at −80 C for biochemical analyzes.

Plant growth parameters

Plant growth was determined by measuring the shoot height (from the root collar to the end of selected branches) at the beginning and end of the experiment. Relative growth rate (RGR) was determined from the mean natural logarithm-transformed plant heights $(\ln H_2) - (\ln H_1)/(t_2 - t_1)$, where t_1 and t_2 are the times 0 and 21 days, respectively as described by Hoffmann and Poorter (2002).

Epidermis and mesophyll autofluorescence using confocal laser scanning microscopy

Autofluorescence in different sections of fresh leaves of epidermal and mesophyll tissue of *V. corymbosum* cultivars was examined early (7 days) using confocal laser scanning microscopy (CLSM, Olympus FV1000, Olympus Co., Tokyo, Japan). Cross-sections of leaf tissues were cut using a double-sided razor blade, mounted in water and then observed. The 488 nm line of an Ar ion laser and 633 nm of a He–Ne laser were used for excitation. A beam splitter (HFT UV/488/633; Olympus Co.) was used to separate excitation from emission and to divide the fluorescence emission into two channels. The chloroplast autofluorescence (633 nm excitation) was visualized at 650–750 nm with a long-pass filter. Phenolic compounds autofluorescence (488 nm excitation) were visualized at 530 nm (Kolb et al. 2001, Agati et al. 2009). Images were processed using image processing software (software FLUOVIEW FV10-ASW v0.200c; Olympus Co.).

Measurement of photosynthetic parameters

Carbon dioxide (CO_2) assimilation was measured in intact fully expanded leaves belonging to the first upper third of non-flowering plants with a portable photosynthesis system (LI-6400, LI-COR Inc., Lincoln, NE) controlling for light (300 µmol photons m^{-2} s^{-1}), temperature (20°C), humidity (80%) and CO_2 (360 ppm) as described earlier by Reyes-Díaz et al. (2011). Measurements were taken on the morning of each collection day. The chlorophyll *a* fluorescence analyzes were determined by using a portable pulse-amplitude modulated fluorimeter (FMS 2; Hansatech Instruments, King's Lynn, UK), exactly as described by Reyes-Díaz et al. (2009, 2010). The maximum quantum yield (Fv/Fm), effective quantum yield (ΦPSII), electron transport rate (ETR) and non-photochemical quenching (NPQ) were calculated according to Maxwell and Johnson (2000).

Photosynthetic pigments

Chlorophyll and carotenoids were extracted from leaves with 100% v/v acetone (HPLC grade) and analyzed according to García-Plazaola and Becerril (1999), using a high-performance liquid chromatography (HPLC) system (Agilent technologies 1200 series, column C-18 Waters spherisorb 5.0 µm ODS1 4.6 × 250 mm). Standards for the pigments Vx, Ax, Zx, neoxanthin (Nx), chlorophyll (Chl) *a*, *b*, *β*-carotene (*β*Ca) and lutein (Lt) were purchased from Sigma-Aldrich (Sigma Chemical Co. St. Louis, MO). The de-epoxidation state of the xanthophyll-cycle pigments (DEPS) was calculated as described by Klem et al. (2015).

Chloroplast isolation and ROS determination using flow cytometry

Chloroplasts were isolated from leaf samples as described by Grabsztunowicz and Jackowski (2012). Later on, intracellular ROS production was determined according to Maxwell et al. (1999) with modifications. The suspension of intact chloroplasts was incubated at 37°C for 30 min with the fluorescent probe chloromethyl 2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) (Invitrogen, Molecular Probes™, Eugene, OR, USA). The chloroplast ROS production was analyzed using flow cytometry (Becton Dickinson™, Becton Dickinson, San Jose, CA, USA). All measurements were taken using an Ar ion laser excited at 488 nm and emitting at 530 nm. The images were processed through the FACSDiva 6.1.3 software (Becton Dickinson™). Autofluorescence of chloroplasts was measured and then a positive control (intact chloroplasts plus H_2DCFDA and 100 µ*M* H_2O_2) and a negative control (intact chloroplasts plus H_2DCFDA without H_2O_2) were assessed (Rabinovitch 2001). Chloroplasts testing positive for ROS fluorescence related to the entire chloroplast population per sample were evaluated.

Determination of ROS in leaf extracts

ROS levels in total leaf extracts were determined as described by Jambunathan (2010) using CM-H_2DCFDA as the ROS-specific probe (Invitrogen, Molecular Probes). The dye CM-H_2DCFDA has absorption at 492–495 nm and maximum emission at 517–527 nm. Fluorescence detection was carried out on a Synergy™ HT multimode microplate reader (Bio-Tek Instruments, Winooski, VT).

Lipid peroxidation and antioxidant activity

As an oxidative stress indicator, the lipid peroxidation (LP) of membranes through malondialdehyde (MDA)

was determined in fresh material using a thiobarbituric acid reacting substances (TBARS) assay according to the modified method of Du and Bramlage (1992). The absorbance was measured at 532, 600 and 440 nm. The radical scavenging activity was determined using the method of oxygen radical antioxidant capacity (ORAC) described by Ou et al. (2001) adapted for a 96-well plate (Roy et al. 2010). Leaves were extracted with 80% ethanol and measured in their antioxidant capacity to inhibit the decline of fluorescence induced by the radical generator 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH). Oxygen radical absorbance capacity values were calculated as the integrated peak area under the decay curve (AUC) between the blank and a sample. The values were expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight.

Total phenols, flavonoids and anthocyanins

Total soluble phenols (TP) contained in ethanolic extracts were determined with Folin-Ciocalteu reagent by the method described by Slinkard and Singleton (1977) using chlorogenic acid as the standard. The extracts were measured spectrophotometrically at 765 nm (UV–VIS Spectrophotometer T80+, PG Instruments, Beijing, China). For total anthocyanins (TA), leaf samples were extracted with acidic ethanol solution (1%) and measured in a spectrophotometer (UV–VIS Spectrophotometer T80+, PG Instruments, Beijing, China) at 530 and 657 nm using a molar extinction coefficient of cyanidin-3-glucoside (Sigma Chemical Co. St. Louis, MO). Results were expressed as milligrams of cyanidin-3-glucoside equivalents per fresh weight (Strack and Wray 1989). Total flavonoids (TFs) were measured by the aluminum chloride colorimetric assay as described by Zhishen et al. (1999) with some modifications using rutin as the standard (Sigma Chemical Co.). Samples were measured spectrophotometrically at 510 nm (UV–VIS Spectrophotometer T80+, PG Instruments). The results were expressed as milligrams of rutin equivalents per gram of fresh weight.

Total soluble UV-B absorbing compounds

The UV-B absorbing capacity of leaves was determined under UV-B conditions as described by Semerdjieva et al. (2003). Briefly, leaf samples from each cultivar were frozen in liquid nitrogen, ground and extracted with methanol for 1 h at room temperature in the dark and centrifuged at 16 000 *g* for 15 min. The supernatant was removed and the pellet was re-suspended with methanol and re-extracted twice for 30 and 10 min, respectively. Supernatants were pooled and stored at 4°C and spectrophotometrically measured from 250 to 400 nm. The UV absorbing compounds (UAC) were normalized to 284 nm according to Semerdjieva et al. (2003) and Clarke and Robinson (2008) and the integration of AUC was performed from 280 to 320 nm. Results were expressed as AUC per mg fresh weight and per leaf area in centimeter square.

Phenylpropanoid profile

Phe and FL composition analyses were performed using HPLC coupled with a mass detector (HPLC-MS) according to Lin and Harnly (2007). Leaves were extracted with methanol 80% v/v. The applied system was an HPLC Shimadzu Prominence coupled to a mass spectrometer MDS Sciex 3200 Qtrap® (Applied Biosystems) with electrospray ionization Turbo VTM at 450°C. The chromatographic separation was done with an RP-C18 Inertsil® ODS-3 column (2.1 × 150 mm, 3 µm; GL Sciences Inc., Tokyo, Japan), an injection volume of 10 µl, 0.2 ml min^{-1} flux, a constant column temperature of 35°C and a mobile phase at different concentration gradients of formic acid 0.1% v/v and methanol. The equipment control and post hoc analysis were done with software Analyst 1.5.1 (AB SCIEX, Ontario, Canada).

Statistical analyzes

All data passed the normality and equality of variance tests after the Kolmogorov–Smirnov test. Data were subjected to a two-way ANOVA where the factors were cultivar and UV-B treatment. A Tukey's significant differences test identified those values with significant differences ($P < 0.05$). Analyzes were performed with Sigma Stat 3.5 (SYSTAT Software Inc.).

Three correlation matrices were performed for each species at each collection point (7, 14 and 21 days) among the three UV-B light irradiances with all parameters. Correlations were calculated from mean data for an accession across all replicates and experiments. The original data are given in the Supplementary section (Table S3). Significance levels were $P < 0.05$. For Pearson's correlation coefficients and *P*-value data, GraphPad Prism 6 was used and a matrix design was generated using Microsoft Office Excel®.

Results

Growth, photosynthetic performance and oxidative stress under contrasting UV-B radiation levels

Plant height was measured to determine RGR. This parameter was reduced by both UV-B doses where the

Table 1. Relative growth rates of plants from two *V. corymbosum* cultivars growing under different UV-B doses for 21 days. Values represent averages of three biological replicates ± SE. Different capital letters indicate significant differences ($P \leq 0.05$) between cultivars for the same treatment. Different lowercase letters show differences ($P \leq 0.05$) between treatments for the same cultivar.

Cultivar	UV-B treatment ($W\,m^{-2}$)	Relative growth rate [mean ± SE ($mm\,day^{-1}$)]
Legacy	Control	0.032 ± 0.006 Ab
	0.07	0.012 ± 0.003 Aa
	0.19	0.009 ± 0.004 Aa
Bluegold	Control	0.038 ± 0.006 Ab
	0.07	0.011 ± 0.005 Aa
	0.19	0.013 ± 0.005 Aa

decrease was around 69% for $0.19\,W\,m^{-2}$ compared with the control treatment (Table 1). Surprisingly, no differences were observed between the Legacy and Bluegold cultivars.

Considering that RGR decreased after UV-B treatments in both cultivars, we decided to investigate whether the UV-B levels would lead to an altered photosynthetic rate (Table 2). Thus, we observed that the CO_2 assimilation rate was different between the two cultivars, with higher values for Legacy. This cultivar displayed a constant CO_2 assimilation rate at all UV-B levels and time periods (Table 2). In contrast, under high UV-B dose, Bluegold showed a strong decrease in the CO_2 assimilation rate from the 7th day, which was statistically significant at 14th and 21st day of treatment compared with the control treatment (Table 2). Interestingly, transpiration rates were not affected by either UV-B or by exposure time to UV-B in either cultivar (Table 2).

The measurement of chlorophyll *a* fluorescence parameters indicated that Fv/Fm, ϕPSII and ETR in Legacy and Bluegold plants did not differ compared with the control plants on either level of UV-B for all time periods (Table 2). Non-photochemical quenching decreased in both cultivars at the high UV-B intensity at all time points, the differences of which were higher at 21 days of treatment. Nonetheless, at low UV-B radiation, only Legacy decreased its values compared with the control (Table 2).

In order to obtain information about the effect of UV-B exposure on carotenoid levels, the concentration of these pigments was measured. In general, the carotenoid pool showed a contrasting trend (Fig. 1). For Legacy, a decrease was found under UV-B radiation from the second week of treatment, whereas Bluegold showed an increase. The levels of βCa and Lt, which is derived from α-carotene, increased around 20% in Bluegold plants under high UV-B doses at 21 days compared with the control treatment (Fig. 1B). Antheraxanthin and Zx decreased in Legacy under high UV-B exposure (Fig. 1A), whereas in Bluegold Ax levels remained constant (Fig. 1B), whereas Vx was increased by UV-B radiation in both cultivars (Fig. 1). The de-epoxidation state (DEPS) indicates the flux of xanthophyll carotenoids toward de-epoxidation of Vx via Ax to Zx. DEPS in Legacy was reduced by the high UV-B radiation at all measured time points compared with the control ($P < 0.05$; Fig. 1). Meanwhile, no changes were found at low UV-B radiation; these values were similar to the control. For Bluegold, a similar reduction in DEPS by UV-B radiation was found with respect to untreated plants ($P < 0.05$), and this occurred at lower UV-B dose than for Legacy. On the other hand, Chl concentrations and ratio were not affected at any time and dose of UV-B radiation of either cultivar (Table S1).

In general, Bluegold presented greater ROS values than Legacy in isolated chloroplasts from UV-B-treated plants compared with untreated plants. The highest ROS level (11-fold higher than control) was exhibited by Bluegold after 7 days of UV-B treatment at low UV-B radiation compared with untreated plants (Fig. 2A). Interestingly, from the 14th to 21st days of UV-B exposure, the differences were smaller (approximately onefold) than at 7 days, but still significant. For Legacy, at the highest UV-B dose, an increase in ROS (twofold) was observed at each time point compared with the control (Fig. 2A). In addition, total ROS produced in leaves did not change at all times points by applying different UV-B treatments in Legacy (Fig. 2B). In contrast, Bluegold showed an increase in ROS accumulation from the 14th day of treatment at the highest UV-B dose. At the end of the experiment, Bluegold showed higher ROS at both UV-B treatments compared with the control (88 and 162%, respectively; Fig. 2B).

The levels of LP for Bluegold increased at 21 days of UV-B treatment (Fig. 2C). In contrast, LP was enhanced in Legacy with the low dose of UV-B radiation after 7 days of treatment, decreasing thereafter (14th and 21st days) at both UV-B doses (Fig. 2C).

Leaf tissue visualization using confocal microscopy and accumulation of UV-B screening compounds

To visualize the impact of UV-B on leaves, we used confocal microscopy to compare Legacy and Bluegold leaf tissue without treatment and after 7 days of exposure to UV-B radiation. Interestingly, bronzing of Legacy leaves subjected to the high dose of UV-B was observed, whereas in Bluegold, this effect was less pronounced. In response to UV-B treatment, Legacy epidermis cells

Table 2. Gas exchange and chlorophyll *a* fluorescence parameters measured in fully expanded leaves from plants of two *V*. *corymbosum* cultivars growing under different UV-B doses. Data are means of three biological replicates ± SE. Different lowercase letters indicate statistically significant differences among treatments for the same cultivar and exposure time (Tukey's HSD at $P \leq 0.05$). Different uppercase letters indicate differences (Tukey's HSD at $P \leq 0.05$) between cultivars for the same exposure time and treatment. Asterisks (*) indicate statistically significant differences at $P \leq 0.001$.

		Legacy			Bluegold		
	T (days)	Control	0.07 W m^{-2}	0.19 W m^{-2}	Control	0.07 W m^{-2}	0.19 W m^{-2}
CO_2 assimilation (μmol CO_2 m^{-2} s^{-1})	7	2.10 ± 0.12aB	2.10 ± 0.10aB	2.03 ± 0.05aB	1.71 ± 0.13bA	1.62 ± 0.15bA	1.27 ± 0.11aA
	14	2.06 ± 0.1aB	2.10 ± 0.03aB	2.00 ± 0.21aB	1.62 ± 0.19bA	1.60 ± 0.19bA	0.55 ± 0.06aA
	21	2.00 ± 0.06aB	2.06 ± 0.13aB	2.01 ± 0.10aB	1.63 ± 0.11bA	1.60 ± 0.10bA	0.66 ± 0.05aA
Transpiration rate (mmol H_2O m^{-2} s^{-1})	7	1.53 ± 0.06aA	1.54 ± 0.11aA	1.58 ± 0.07aA	1.40 ± 0.06aA	1.50 ± 0.04aA	1.50 ± 0.04aA
	14	0.83 ± 0.00aA	0.86 ± 0.01aA	0.86 ± 0.01aB	0.84 ± 0.01bA	0.82 ± 0.01 abA	0.8 ± 0.01aA*
	21	0.83 ± 0.01aA	0.82 ± 0.02aA	0.81 ± 0.00aA	0.86 ± 0.01bA	0.84 ± 0.00 bA	0.8 ± 0.01 aA
Fv/Fm	7	0.84 ± 0.01aA	0.87 ± 0.01aA	0.84 ± 0.01aA	0.86 ± 0.01aA	0.85 ± 0.01aA	0.83 ± 0.00aA
	14	0.83 ± 0.00aA	0.86 ± 0.01aA	0.86 ± 0.01aB	0.84 ± 0.01bA	0.82 ± 0.01abA	0.80 ± 0.01aA
	21	0.83 ± 0.01aA	0.82 ± 0.02aA	0.81 ± 0.00aA	0.86 ± 0.01bA	0.84 ± 0.00bA	0.80 ± 0.01aA
ΦPSII	7	0.24 ± 0.01abA	0.22 ± 0.01aA	0.27 ± 0.01bA	0.23 ± 0.01aA	0.23 ± 0.0 aA	0.22 ± 0.01aA
	14	0.23 ± 0.01aA	0.23 ± 0.02aA	0.26 ± 0.02aA	0.2 ± 0.00aA	0.23 ± 0.01aA	0.22 ± 0.02aA
	21	0.26 ± 0.01aA	0.26 ± 0.01aA	0.23 ± 0.01aA	0.23 ± 0.01aA	0.24 ± 0.01aA	0.18 ± 0.01aA
ETR	7	30.3 ± 0.93abA	27.34 ± 0.77aA	33.9 ± 1.65bB	28.8 ± 1.24aA	28.4 ± 0.31aA	27.6 ± 1.58aA
	14	29.01 ± 1.35aA	29.34 ± 0.48aA	32.6 ± 2.77aA	27.9 ± 0.33aA	29.0 ± 1.45aA	27.7 ± 2.22aA
	21	33.07 ± 1.42aA	33.02 ± 1.52aA	28.95 ± 1.2aA	29.0 ± 1.21aA	29.7 ± 1.57aA	23.2 ± 1.53aA
NPQ	7	3.26 ± 0.04aA	2.73 ± 0.1aA	3.09 ± 0.26aA	2.85 ± 0.11aA	2.48 ± 0.06aA	2.73 ± 0.06aA
	14	3.26 ± 0.12cA	2.26 ± 0.23bA	1.75 ± 0.18a*A	2.59 ± 0.13abB	3.07 ± 0.09bB	2.35 ± 0.05aB
	21	3.06 ± 0.11bA	1.79 ± 0.47aA	1.47 ± 0.1aA	2.79 ± 0.08bA	2.78 ± 0.11bB	1.58 ± 0.17aA

exhibited higher levels of green fluorescence, corresponding to fluorescence of phenolic compounds, than Bluegold epidermal cells (Fig. S2). Green fluorescence was 27-fold higher than controls in Legacy, whereas in Bluegold this was 4.3-fold (Table S2). Red fluorescence, resulting from chlorophyll autofluorescence, is equally increased by UV-B treatment, but only slightly in both Legacy and Bluegold (1.3- and 1.5-fold, respectively) (Table S2). A disturbance was also observed in the autofluorescence of chloroplasts at the mesophyll level in Bluegold, suggesting a disruption in chloroplast integrity (Fig. S2B). Contrarily, this phenomenon was not observed in Legacy leaves (Fig. S2A).

Antioxidant activity in leaves after UV-B radiation treatment

To better understand the metabolic responses of plants exposed to UV-B radiation, several secondary metabolites were quantified. A significant interaction between cultivar and UV-B dose was observed for ORAC in Legacy at 14 and 21 days (Fig. 3A). There was a mild increase in ORAC in Legacy at 7 days under the highest dose of UV-B radiation, and after 14 days this increase was significantly higher with both UV-B radiation levels compared with the control. On the other hand, for Bluegold no change caused by the UV-B exposure was observed (Fig. 3A). In general, it was observed that UV-B radiation did not influence SOD activity in Legacy plants. However, in Bluegold plants an increase in SOD activity was observed at the highest UV-B level on the 7th and 21st day (Fig. 3B).

Accumulation of secondary metabolites involved in UV-B responses

As compared with controls at the same time point, TP in Legacy increased as UV-B dose increased over the whole study period (Fig. 4A), with the highest TP accumulation, 1.7-fold over controls, being observed at 21 days under the highest UV-B dose. In contrast, Bluegold exhibited only minor changes in TP in response to UV-B, with the exception being on the 14th day at the highest UV-B dose, where a 1.7-fold increase in TP was observed (Fig. 4A).

The two cultivars displayed differential responses in terms of TA concentration under UV-B treatment (Fig. 4B). For Legacy, no change in TA was observed at either UV-B dose until 21 days of exposure, when TA levels were found to have decreased. In contrast, decay in TA levels was revealed in Legacy plants at 21 days of exposure to UV-B radiation (Fig. 4B). However, for Bluegold the opposite changes were observed over time: a significant increase in TA from the 14th to 21st days was observed at the highest UV-B dose (Fig. 4B).

In terms of TF accumulation, no difference was detected in Bluegold compared with the control at any UV-B treatment or any exposure time (Fig. 4C). In Legacy

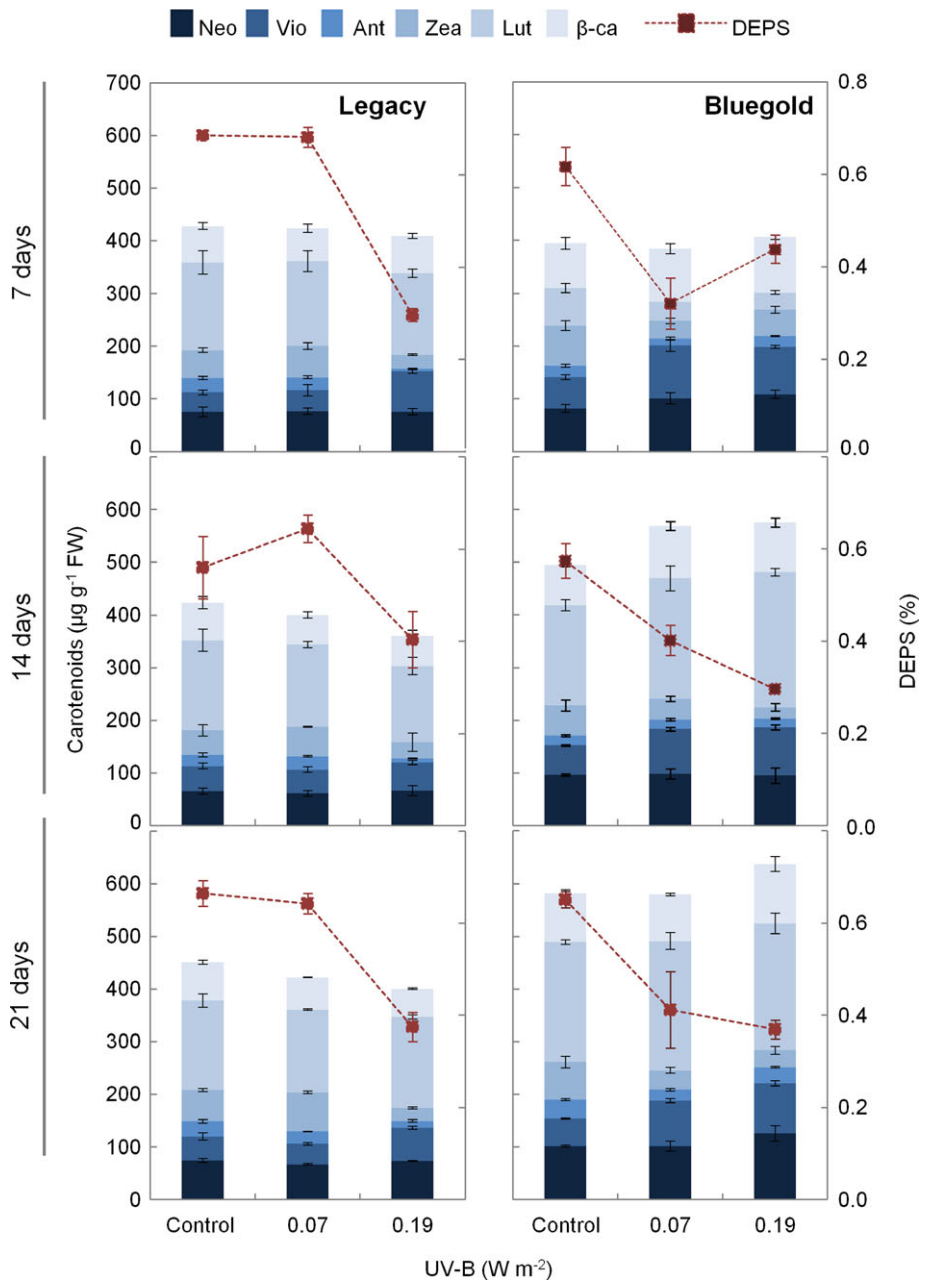

Fig. 1. Carotenoid pigments concentration (µg g^{-1} FW) and the nominal de-epoxidation state (DEPS) of the xanthophyll cycle pigments in leaves of plants from two highbush blueberry cultivars growing under control and UV-B radiation conditions. Values represent the average of three replicates ± SE. Ant, antheraxanthin; DEPS, de-epoxidation state of the xanthophyll-cycle pigments; Lut, lutein; Neo, neoxanthin; Vio, violaxanthin; Zea: zeaxanthin, *β*-ca: *β*-carotene.

a clear increase in TF was observed, around 71% at the highest UV-B dose at 7 days of treatment, then levels decreased by around 31% at 21 days with both UV-B doses (Fig. 4C).

Ultraviolet absorbing compounds showed an increase only for Legacy plants at the highest UV-B dose at 7 and 14 days of treatment compared with the control (Fig. 4D). The major differences occurred at 14 days when there was an increase of about 92%. At 21 days, AUC values in Legacy were lower, and were similar to those found in Bluegold across the experimental period (Fig. 4D).

Additionally, a phenolic profile at the end of the experiment was carried out considering only de novo

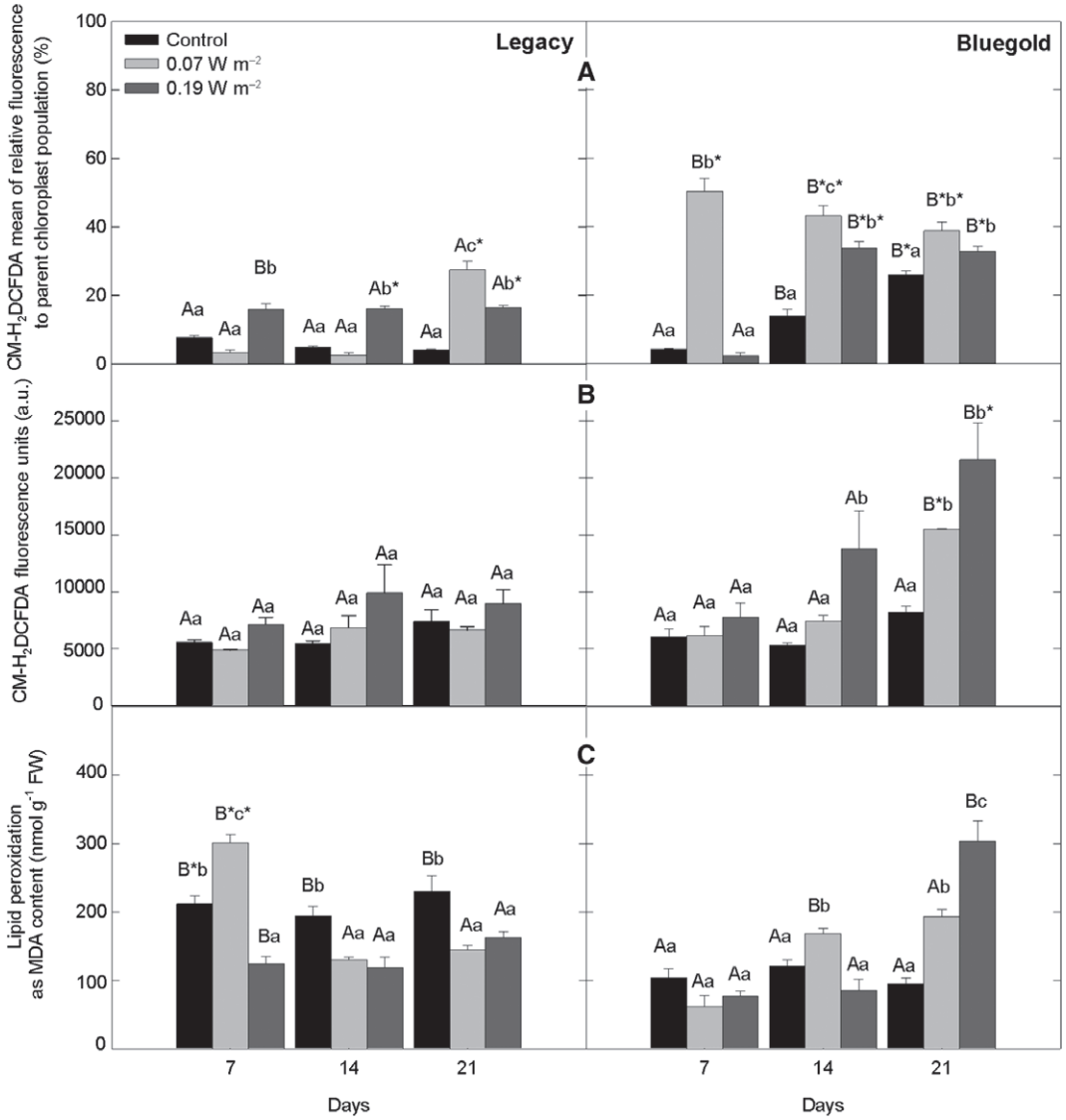

Fig. 2. ROS generation in chloroplasts of *V. corymbosum* plants under UV-B radiation conditions. Chloroplasts were stained with 10 μ*M* of CM-H_2CDFDA dye and samples were incubated for 30 min at 37°C. (A) ROS production in chloroplasts under UV-B treatments. (B) Total ROS. (C) Lipid peroxidation as malondialdehyde (MDA) concentration. All values represent the average of three replicates ± SE. Different lower case letters indicate statistically significant differences (Tukey's HSD at $P \leq 0.05$) among treatments for the same cultivar and time. Different uppercase letters indicate differences (Tukey's HSD at $P \leq 0.05$) between cultivars for the same treatment and time.

phenolic compounds induced by the UV-B radiation treatments (Table 3). These compounds were not found in the control plants. A differential phenolic profile between the two cultivars was found, where Legacy showed a wider variety of induced compounds than the Bluegold plants under UV-B treatments (Table 3). Legacy synthesized mostly flavonol glycosides, such as quercetin-rhamnoside, kaempferol-glucoside and myricetin-xyloside. In general, all the induced compounds absorb strongly in the UV-B and UV-A ranges (Table S4).

Correlation analysis between physiological and metabolic parameters

To assess the level of association between the measured variables, Pearson's correlation coefficient was calculated for all pairs of measured parameters across the three UV-B radiation conditions (Fig. 5). At a level of 5% significance, a total of 289 correlations were found, of which 171 were positive and 118 were negative. Differences were observed between the matrix correlations of

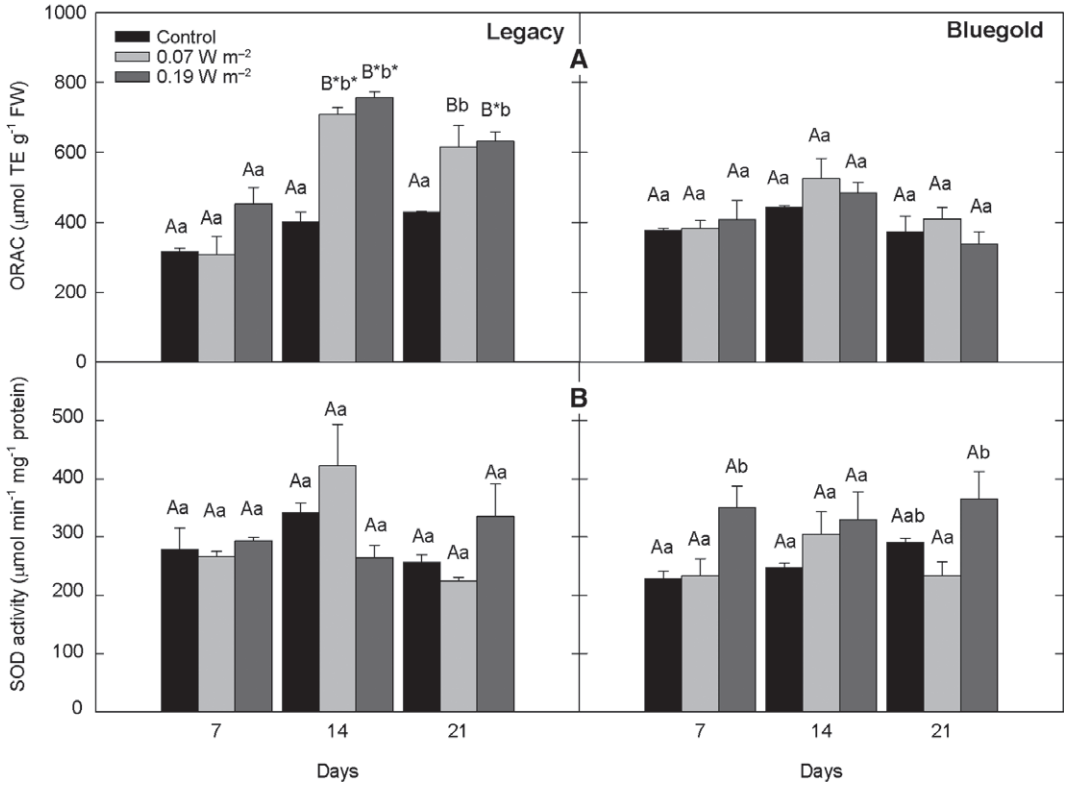

Fig. 3. Antioxidant capacity of leaves of *V. corymbosum* plants grown under different UV-B treatments. (A) Oxygen radical activity (ORAC) measured as Trolox equivalents (TE) and (B) Enzymatic antioxidant activity as superoxide dismutase (SOD). All values are the average of three replicates ± SE. Different lowercase letters indicate statistically significant differences among treatments for the same cultivar and exposure time. Different uppercase letters indicate differences (Tukey's HSD at $P \leq 0.05$) between cultivars for the same exposure time and treatment.

the two cultivars (Fig. 5). As noted, Legacy activated a non-enzymatic antioxidant system at the onset of stress. As evidence, flavonoids were positive strongly correlated with total ROS and chloroplast ROS on the 7th day (Fig. 5A) and phenols strongly correlated with phenols with total ROS and chloroplast ROS on the 14th day (Fig. 5C). A positive correlation between ROS levels and phenolic compounds was observed only on the 21st day in Bluegold. After 7 days of treatment, negative correlations were found between LP and ROS produced in the chloroplast, total ROS levels, TF and Vx content, as well as ϕPSII, ETR in Legacy (Fig. 5A). In the following days this behavior was not so evident, and most of the LP correlations were positive (Fig. 5C, E). Interestingly, Bluegold plants exhibited a different behavior. In this cultivar, it was observed that most of the physiological parameters at 21 days, such as CO_2 assimilation and Fv/Fm, were negatively correlated with many of the other parameters, including LP, total ROS, TP, TA, Nx and Lt levels (Fig. 5F). This highlights the sensitivity of Bluegold to UV-B radiation in comparison with Legacy's tolerance to the same treatment.

Discussion

This study applied simulated UV-B radiation conditions on *V. corymbosum* cultivars for 3 weeks. We observed that although both cultivars were affected by UV-B radiation in terms of shoot growth, their plant performance was completely different. Previous studies have suggested that UV-B radiation alone and combined with manganese toxicity affects stem elongation in highbush blueberry (Rojas-Lillo et al. 2014). It has also been demonstrated that plant growth is strongly affected by UV-B radiation (Teramura and Sullivan 1994, Krizek et al. 1997, Conner and Neumeier, 2002, Kumari et al. 2009, Zhu and Yang 2015). One of the reasons for the reduced plant growth following UV-B exposure could be the reduction in photosynthetic performance as well as the amount of assimilates as observed in grape (Kolb et al. 2001) and rice (Mohammed and Tarpley 2010). In cucumber plants, an increase in stem elongation and growth under UV-B exclusion has been observed, showing that growth can be affected even at ambient levels of UV-B (Krizek et al. 1997, Kanungo et al. 2013). These effects are generally mild and are more pronounced

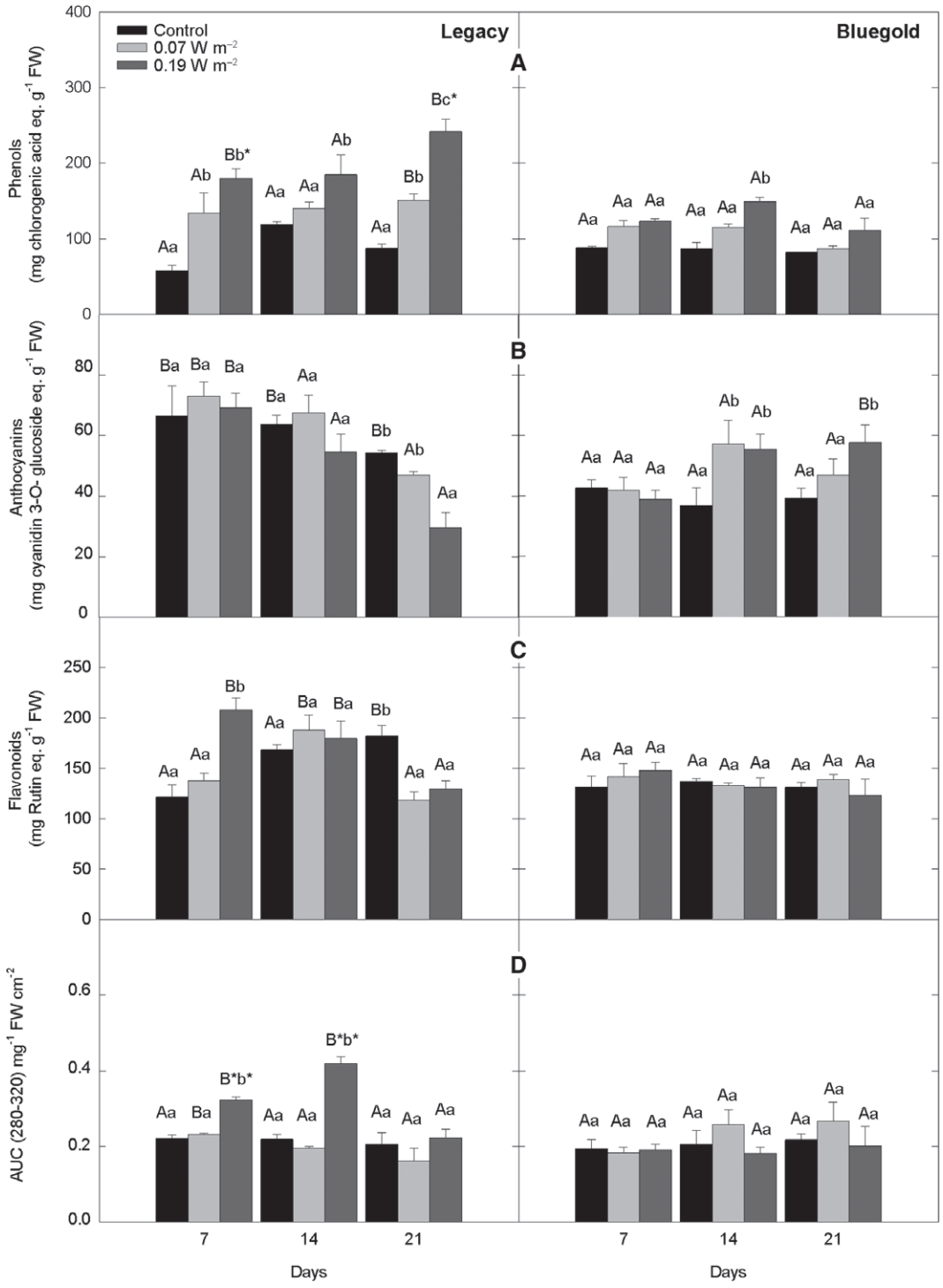

Fig. 4. Total phenols (A), anthocyanins (B), flavonoids (C) and UV-B absorbing capacity (D) from leaves of *V. corymbosum* cultivars grown under different UV-B light treatments at different time periods, measured as chlorogenic acid, cyanidin-3-glucoside, rutin equivalents and area under the curve (AUC) integrated between 280 and 320 nm, respectively. All values correspond to the average of three replicates each ± SE. Different lower case letters indicate statistically significant differences among treatments for the same cultivar and exposure time. Different upper case letters indicate differences (Tukey's HSD at $P \leq 0.05$) between cultivars for the same exposure time and treatment.

Table 3. Compounds induced by UV-B radiation in leaves of *Vaccinium corymbosum* plants growing under UV-B treatments for 21 days. Treatments: low, 0.07 W m^{-2} UV-B; high, 0.19 W m^{-2} UV-B. Details of compound characterization can be found in Table S4.

Cultivar	Class	ID	UV-B treatment (W m^{-2})	
			0.07	0.09
Legacy				
	Phenolic acid	Dicaffeoylquinic acid	+	−
		Caffeoylshikimic acid	+	+
		5-*O*-Feruoylquinic acid	+	+
	Flavonol	Kaempferol glucoside (sodium adduct m/z 535)		+
		Kaempferol glucoside (sodium adduct m/z 419 Kaempferol Arabinoside)	+	−
		Kaempferol 3-*O*-(6″-*O*-malonyl) glucoside	−	+
		Myricetin-3-xyloside	+	−
		Quercetin-3-rhamnoside	+	+
		Ishoramnetin-3-rutinoside	+	−
		Ishoramnetin-3-glucoside	+	−
		Kaempferol-7-O-rhamnoside	−	+
	Flavonone	Pentahydroxyflavanone-A hexoside	+	+
	Flavan 3 ol	Epicatechin	+	+
		Gallocatechin	+	+
Bluegold				
	Phenolic acid	5-*O*-Feruoylquinic acid	+	−
		Myricetin-3-xyloside	+	−
	Flavonol	Isorhamnetin	+	+
		Prodelphynidin B3	+	−

in herbaceous species than in woody perennial plants (Giordano et al. 2004). Nonetheless, under a daily UV-B course simulation, we found that in *V. corymbosum* UV-B reduced stem growth (>60%; Table 1), with both cultivars being equally affected regardless of the UV-B dose. We also observed that CO_2 assimilation in Legacy was not directly affected by UV-B exposure and therefore it cannot be associated with reduced plant growth; by contrast, in Bluegold a direct relationship was found between CO_2 assimilation and growth. In addition, CO_2 assimilation decayed at the end of the experiment in Bluegold. This decrease in CO_2 assimilation rates has been associated with damage to the light harvesting complexes, disruption of thylakoid membrane integrity, inactivation of RuBisCO or a change in stomata conductance (Takeuchi et al. 2002, Kosobryukhov et al. 2015).

It is known that UV-B can increase ROS production in UV-B-sensitive plants, affecting the photosynthetic apparatus (Strid et al. 1994, Agrawal and Mishra 2009, Kataria et al. 2014). The plant low capacity to screen this radiation from the photosynthetic tissues is an indicator of UV-B sensitivity (Middleton and Teramura 1993). Consequently, the evaluation of this response on isolated chloroplasts gives information about the incoming intensity of UV-B reaching these organelles (Hideg et al. 2013). In our experiment, Bluegold increased H_2O_2 production in chloroplasts sooner than Legacy under low UV-B radiation (Fig. 2A). Thus, we believe that the intake of this radiation to the inner cell layers was stronger in the first days of treatment in Bluegold. In fact, higher levels of chloroplast ROS were correlated negatively with increased LP ($r = -0.91$) in Legacy (Fig. 5), whereas Bluegold exhibited a positive correlation with LP ($r = 0.97$) and total ROS at 21 days. The LP increase in Bluegold indicated higher sensitivity to UV-B during prolonged exposure (Fig. 2C). Furthermore, UV-B sensitivity in this cultivar may be associated with a decrease in CO_2 assimilation (Table 2). Meanwhile, such physiological parameters were not affected in Legacy plants (Table 2).

Increases in the scavenging parameters under UV-B radiation treatments have been extensively discussed in different plant species (Hideg et al. 2013). The results obtained in the present study suggest that Bluegold counteracts the ROS accumulation with UV-B-enhancing SOD activity (Fig. 3B). Nonetheless, this response seemed to be insufficient to counteract the harmful effects of UV-B reflected in the increase in LP at the end of experiment.

Xanthophyll cycle compounds could be involved in the photosynthetic apparatus protection against UV-B radiation; however, this protection is poorly understood (Yang et al. 2007, Moon et al. 2011). In our study, we found a differential response to UV-B radiation in both cultivars with respect to xanthophyll pigments. Lt levels were increased in Bluegold with the highest UV-B dose, which is relevant because of the involvement of

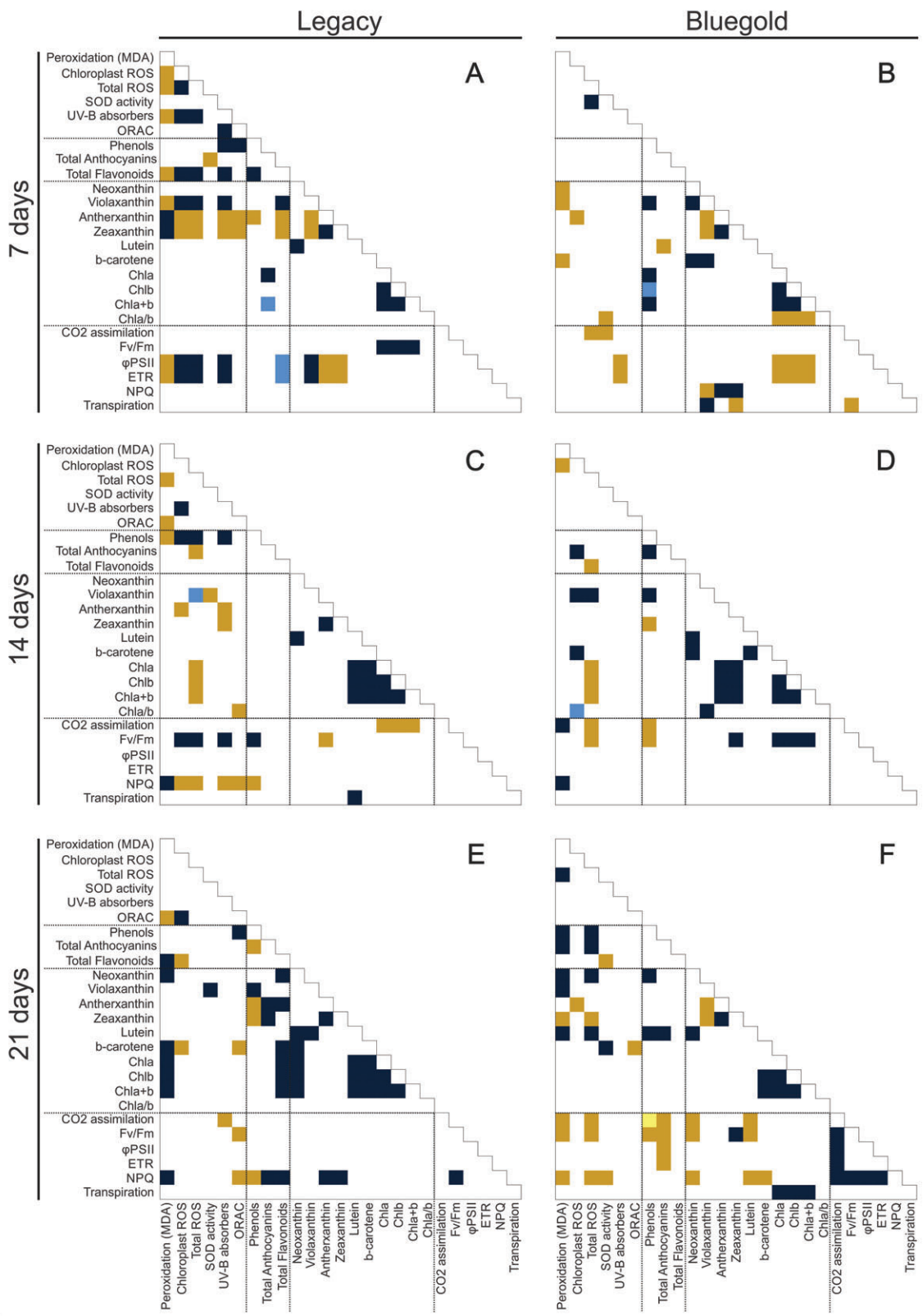

Fig. 5. Continued.

this carotenoid in NPQ. This was demonstrated in the *npq1* mutant of *Arabidopsis*, where the accumulation of Lt in the absence of Zx restored the NPQ (Li et al. 2009). In this context, our results did not support this assumption because Bluegold decreased NPQ at the highest UV-B dose at the end of the experiment. Bluegold also increased βCa at the end of the experiment. Yet despite this cultivar showing an increase in Lt and βCa, these compounds were not correlated with either NPQ or ORAC. Therefore, we suggest that in Bluegold plants these increases seem to be a UV-B stress response and not a UV-B resistance mechanism. The reduction of DEPS at the high UV-B radiation in Legacy and at both UV-B levels in Bluegold (Fig. 1) suggests that Vx de-epoxidase was inactivated by UV-B radiation, leading to an accumulation of Vx in both cultivars. In fact, the inhibition of Vx de-epoxidation by UV-B radiation has been reported previously in *Pisum sativum* (Pfündel et al. 1992), *Cucumis sativus*, *Solanum lycopersicum* and *A. thaliana* (Moon et al. 2011). However, these last authors reported that an increase in VDE/ZEP ratio in UV-B-treated plants indicated greater enzyme activity for de-epoxidation of Vx to Zx than for Zx-epoxidase, which was not consistent with the NPQ suppression by UV-B (Moon et al. 2011). Although a central role of pH in thylakoid lumen acidification because of electron transport has been hypothesized to explain xanthophyll cycle activation and NPQ, our results indicate that ETR was not affected in either cultivar by UV-B radiation (Table 2). This confirms our assumption about the possible inhibition of Vx de-epoxidase by UV-B exposure.

Another important response to UV-B radiation is the increase in phenylpropanoid compounds (Caldwell et al. 1983, Li et al. 1993, Landry et al. 1995, Hectors et al. 2007, Jenkins 2009, Randriamanana et al. 2015). Both flavonoids and hydroxycinnamic derivatives are widely reported to be sunscreens (Rozema et al. 1997, Fischbach et al. 1999, Tilbrook et al. 2013). Our findings indicate that flavonoid accumulation is responsive to UV-B, increasing in concentration in Legacy at the highest UV-B dose at 7 days (Fig. 4C). However, we observed a null response in this cultivar with respect to anthocyanins (Fig. 4B). Anthocyanins have been previously reported as being induced by stress factors in *V. corymbosum* (Inostroza-Blancheteau et al. 2014, Rojas-Lillo et al. 2014, Yañez-Mansilla et al. 2015, Reyes-Díaz et al. 2016). In our case, these cultivars did not show a clear induction of anthocyanins under UV-B radiation. Bluegold did show an increase of these metabolites at 14 and 21 days, but this response seems to be associated with stress conditions and not particularly with a UV-B photoprotective mechanism, because this cultivar was damaged by UV-B treatments.

The accumulation of UV-B absorbing compounds (UAC), a common response strategy to UV-B in plants, was induced in our experiment. Legacy showed an increase in these compounds earlier (7 days), maintaining higher levels than the control at the highest UV-B treatment. A contradictory behavior was found in Bluegold, where UAC did not change during the exposure to UV-B radiation (Fig. 4D).

Flavonols can contribute as a UV-B radiation exclusion mechanism. Our findings suggest that Legacy has a higher pool and wider variety of UV-B photoprotective compounds than Bluegold, biosynthesizing mostly flavonol glycosides, which could afford some advantages in the avoidance of UV-B stress. All these molecules strongly absorb in the UV-B range (280–320 nm), which is consistent with other reports (Caldwell et al. 1983, Emiliani et al. 2013). The glycosylation of these molecules also reduces their antioxidant capacity, giving the molecule greater stability and solubility (Pietta 2000). In fact, it is reported that a large number of these compounds are accumulated in vacuoles of epidermis cells (Solovchenko and Merzlyak 2008), where it is suggested they act as photoprotective compounds under UV radiation (Merzlyak et al. 2008).

According to Hideg et al. (2013) and based on our results about increased levels of ROS, LP and reduction in CO_2 assimilation, Bluegold could be considered as developing a distress (a strong stress event following an unfavorable change in environmental conditions leading to metabolic damage), indicating that this cultivar is sensitive to UV-B radiation. By contrast, Legacy seems to be a UV-B-resistant cultivar because of a better photosynthetic and antioxidant performance.

Conclusions

The results of this study showed that, under a simulated daily course of UV-B radiation, the cultivars behaved

Fig. 5. Correlation matrices based on Pearson's correlation coefficients between antioxidative metabolism responses, secondary metabolites, pigments and photosynthetic parameters of Legacy and Bluegold cultivars. Correlation was calculated at three points 7 (A and B), 14 (C and D) and 21 (E and F) days and three UV-B radiance (0, 0.07 and 0.19 W m^{-2}). Pearson coefficient that are significant at $P < 0.05$ are indicated by dark and light shading, which means strong (0.67 to 1.0) and weak (0.34 to 0.66) correlation, respectively. Positive and negative correlations are distinguished by blue and yellow colors, respectively.

differently under UV-B stress: while Legacy was UV-B resistant, Bluegold was UV-B sensitive. It is worth noting that Legacy revealed a combined strategy to cope with the intake of UV-B radiation, which is primarily better oriented to photoprotection (avoidance) and then to tolerate the impact of this radiation. This strategy was shown to be dose-dependent, and it is triggered early under high UV-B radiation in this cultivar. Interestingly, the behavior of Legacy under a high UV-B dose showed an initial increase in its UV-B absorbing capacity during the first week of treatment, where flavonoids seemed to have the main role. From the second week, plants responded by reprogramming their metabolism, thereby increasing their antioxidant capacity and augmenting the biosynthesis of phenolic molecules. However, the capacity of Legacy to synthesize a wider variety of phenolics than Bluegold may also be involved in increasing its efficiency to tolerate high doses of UV-B. In general, the capacity of Legacy to respond early to UV-B by reprogramming its metabolism is the main difference with the sensitive cultivar Bluegold, which seems to react later, leading to metabolic damage.

Author contributions

A. L.-E., M. R.-D and M.A. designed and coordinated the experiments. A. L.-E., M. R.-D., M. A. and C. I.-B. formulated the manuscript and A. L.-E., M. R.-D, M. A., C. I.-B, and A. N.-N revised and corrected it. P. A. performed the UV-B irradiance measurements. A. L.-E and M. R.-D carried out the physiological, biochemical and metabolic analyzes. M. M. and A. N. N. performed the statistical analysis.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Daily course of UV-B simulation (280–320 nm) in greenhouse experiment.

Fig. S2. Confocal laser microscopy images from leaves of *Vaccinium corymbosum* cultivars under UV-B treatment for 7 days.

Table S1. Chlorophyll concentration detected by HPLC-DAD of two highbush blueberry cultivars under control and UV-B radiation conditions.

Table S2. Relative total leaf fluorescence intensity on *Vaccinium corymbosum* cultivars exposed to UV-B radiation for 7 days.

Table S3. Correlation matrices based on Pearson correlation coefficients, between oxidative metabolism parameters, pigments and photosynthetic parameters of Legacy and Bluegold blueberry varieties, in 7, 14 and 21 days of three UV-B irradiances.

Table S4. Compounds induced by UV-B radiation and detected by HPLC MS/MS in *V. corymbosum* cultivars under UV-B radiation at the end of the time point experiment.

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